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ORIGINAL ARTICLE

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Measurement of serum vitamin B12-related metabolites in newborns: implications for new cutoff values to detect B12 deficiency

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ABSTRACT

Objective: Our aim was to determine the prevalence of maternal and neonatal vitamin B12 (vit-B12) and folate deficiencies, a new cutoff value of serum vit-B12 in newborns using vit-B12-related metabolites and also cutoff values of homocysteine (Hcy), propionyl (C3) carnitine, and methyl malonic acid (MMA) in newborns using a vit-B12 cutoff value of 200 pg/mL.

Methods: Healthy pregnant women (without iron deficiency) and 98 healthy, term, singleton babies were included. Blood samples were obtained from women 0–8 h before birth and from cord blood during birth for hemogram and to measure serum vit-B12, folate, and Hcy levels. Maternal and cord blood serum vit-B12 levels were classified as low < 200 pg/mL, marginal 200–300 pg/mL, and normal ≥ 300 pg/mL. Neonatal urine MMA levels were analyzed in mothers with a vit-B12 concentration < 300 pg/mL. C3 carnitine levels of newborns were acquired from extended newborn screening. Receiver operating characteristics curve (ROC) analysis was used for serum vit-B12, urine MMA, C3 carnitine, and Hcy.

Results: Of total, 98 pregnant women (28.6 ± 5.5-year-old) and 98 newborn were included. Vit-B12 level was lower than 300 pg/mL in 93% of the pregnant women and 61% of cord blood samples. Folate deficiency was not found in either group. There was statistically significant negative correlation between baby C3 carnitine, cord blood folate ($r = -0.265$, $p = .008$) and cord blood vit-B12 ($r = -0.220$, $p = .029$). In backward stepwise linear regression analysis, maternal vit-B12 level exerted the most marked effect on cord blood vit-B12 level (adjusted $R^2 = 0.457$). In ROC analysis, the Hcy cutoff value was 4.77 μmol/L (68.4% sensitivity, 58.3% specificity, $p = .012$) for the detection of vit-B12 deficiency.

Conclusion: Vit-B12 deficiency remains an important health issue for pregnant women and newborns. Our study revealed a cutoff value for Hcy for the detection of nutritional vit-B12 deficiency that could be used in practice for newborns.

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Folate; homocysteine; newborn; urine methylmalonic acid; vitamin B12 deficiency

Introduction

Rapid physical and neurologic developments occur during both the fetal and neonatal periods. Thus, deficiencies of vitamin B12 (vit-B12) and folate, necessary for synthesis of DNA, RNA, lipids, and proteins pose a significant problem [1]. Effects of these vitamin deficiencies on neurological development include motor development retardation and cognitive dysfunction due to delay in myelination, demyelination, dysmyelination, and/or axonapathy [1]. In addition low birth weight may impair physical development [2]. In vit-B12 and folate deficiency, these clinical consequences usually develop due to elevation of homocysteine (Hcy), methyl malonic acid (MMA), and propionyl (C3) carnitine concentrations or absence of methionine in blood [3].

The World Health Organization (WHO) has defined cutoff values for vit-B12 deficiency of below 221 pmol/L (148–221 pmol/L marginal B12 and < 148 pmol/L B12 deficiency) [4]. However, these values did not take

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Table 1. Comparison of maternal age, parity number, sex of the baby and anthropometric measurements with low, marginal, and normal vitamin B12 levels in cord blood.

	B12 level in cord blood (pg/mL)			
	<200 ($n = 25$)	200–300 ($n = 35$)	300($n = 38$)	p
Maternal age (years) (mean ± SD)	27.0 ± 4.5	30.8 ± 6.3	27.5 ± 4.7	**.026**[a]
Type of delivery, *n* (%)				
Vaginal birth	3 (12)	10 (40)	12 (48)	.195
Cesarean Birth	22 (30)	25 (34)	26 (36)	
Parity				
Parity 0	10 (25)	14 (35)	16 (40)	.555
Parity 1	12 (29)	12 (30)	17 (41)	
Parity ≥ 2	3 (18)	9 (53)	5 (29)	
Sex of the baby, *n* (%)				.141
Female	12 (25)	21 (45)	14 (30)	
Male	13 (26)	14 (27)	24 (47)	
Weight of baby (g) (mean ± SD)	3370.4 ± 463.4	3148.9 ± 459.7	3397.1 ± 451.2	.055
Length of baby (cm) (mean ± SD)	48.2 ± 2.2	47.9 ± 1.8	48.7 ± 1.6	.165
Ponderal index (mean ± SD) (weight (g) × 100/(height, cm)^3)	3.1 ± 0.5	2.9 ± 0.4	2.9 ± 0.3	.095
Head circumference of baby (cm) (mean ± SD)	34.7 ± 1.4	33.76 ± 0.7	34.3 ± 0.8	**.003**[b]

[a]p value is given for the comparison of normal (>300 pg/mL) and marginal zone (200–300 pg/mL) groups.
[b]p value is given for the comparison of deficient (<200 pg/mL) and marginal zone (200–300 pg/mL) groups.

into account age groups. Scolamiero et al. [5] suggested a new cutoff value for vit-B12 for newborns, but their study cohort consisted of only seven B12-deficient neonates with C3 carnitine elevation detected by extended newborn screening tests. Thus, it has been suggested that serum vit-B12 level alone is not enough for detecting vit-B12 deficiency [3, 6–8] and at least two vit-B12-related metabolites should be evaluated for reliable detection of vit-B12 deficiency [7,8].

Hannibal et al. [3] designated cutoff values for both B12 and associated biomarkers (holo-transcobalamin, Hcy, and MMA) classifying B12 levels as high, sufficient, low (subclinical), possible deficiency and probable deficiency (where clinical symptoms emerge). However, these limit values did not take subject age into account. This meta-analysis was a call for attention about the absence of a study evaluating all vit-B12 related biomarkers together: serum B12; folate; Hcy; MMA; and C3 carnitine.

In this study, our aim was to determine: (a) vit-B12 and folate deficiencies in pregnant women and newborns; (b) a cutoff value of serum vit-B12 for newborns by measuring multiple vit-B12 related metabolites (Hcy, C3 carnitine, and urine MMA); and (c) cutoff values of MMA, Hcy and C3 carnitine for newborns in relation to measured vit-B12.

Materials and methods

This study was conducted on pregnant women and their term-singleton babies who were referred to an obstetrics and gynecology outpatient clinic for delivery between December 2009 and January 2010. The subjects included 98 pregnant women with no health problems and no iron deficiency and 98 healthy, term, singleton babies. Teenage pregnancies and women with acute or chronic diseases were excluded. Pregnant women were informed about the objective of the study and informed consents were obtained prior to recruitment. The study was approved by the local ethical committee (no. 2008/3003).

Data collection

Data about the age of mother, consumption of complementary folic acid and vit-B12 (at least 3 months) during pregnancy, parity of newborn and weekly consumption of animal and vegetable products (red and white meat, fish, egg, milk/cheese/yoghurt, and vegetable/fruit) were collected from the women before delivery. Anthropometric measurements (weight (g), length (cm), and head circumference (cm)) of newborns were obtained from their birth cards.

Urine and blood sampling and analysis

Blood samples were obtained from pregnant women 0–8 h before birth and from cord blood during birth. EDTA blood was analyzed within 0–4 h for the complete blood count using impedance/flow cytometry/photometry. Samples in plain tubes were allowed to clot and then centrifuged. The samples were kept at 2–8 °C and analyzed within a maximum of 2 d. Serum vit-B12 was measured using electrochemiluminescence (Roche Diagnostics GmbH, Mannheim, Germany) (an intra-assay coefficient of variation (*CV*) of 8.7%, an interassay *CV* of 9.5%). The reference interval for serum vit-B12 level varies by country and region [9]. For the purposes of this study, we classified vit-B12

level as follows: low < 200 pg/mL; marginal 200–300 pg/mL; and normal ≥ 300 pg/mL. Folate assessment was performed using an Elecsys 170 analyzer (Roche Diagnostics GmbH, Mannheim, Germany; intra-assay *CV* of 5.6%, interassay *CV* of 13.8%) with a reference interval 3.1–17.5 ng/mL. The competitive immunoassay method was used for Hcy levels (Siemens Medical Solutions Diagnostics, Los Angeles, CA; intra-assay *CV* of 9.1%, interassay *CV* of 9.9%) with a reference interval 5–12 μmol/L.

Urine samples of newborns of those mothers with a vit-B12 concentration < 300 pg/mL were obtained between 0 and 4 d after birth, kept at −80 °C and measured in batches. A gas chromatography/mass spectrometry method was used for the assessment of urine MMA. Any level of MMA excretion in urine was accepted as pathological. C3 carnitine levels were acquired from the extended newborn screening results.

Statistical analyses

Statistical Package for the Social Sciences, version 15 (SPSS, IBM Inc, Chicago, IL) was used for the statistical analyses. Results are given as mean ± standard deviation (SD) or median [range]. Normality was assessed using the Kolmogorov–Smirnov test. The Chi-square test was used for categorical variables and Student's *t*-test for continuous variables. The Mann–Whitney *U* test was used for nonparametric continuous variables, while the one-way ANOVA test was used for normally distributed continuous variable comparison between more than two groups. Spearman and Pearson's correlation analysis were used for nonparametric and parametric distributed parameters, respectively. The linear regression model was applied using the backward stepwise method with cord blood serum vit-B12 level as the dependent variable and independent variables were maternal age, maternal vit-B12 and folate concentration, weight of newborn, serum Hcy and C3 carnitine. A p value < .05 was accepted as significant.

Using the known cutoff values of vit-B12 metabolites (urine MMA cut off value for vit-B12 deficiency = 0 mmol/molcreatinine, C3 carnitine cutoff value for vit-B12 deficiency = 3.16 μmol/L), receiver operating characteristics (ROC) curve analysis was applied for vit-B12. We did not analyze ROC curve by Hcy due to the absence of pathological Hcy concentrations in cord blood samples. Also, ROC curve analyses were applied for Hcy and C3 carnitine using a vit-B12 cutoff value of 200 pg/mL (221 pmol/L). When the type-1 error level was below 5% in the area under the curve (AUC) analysis, the diagnostic value of the test was considered statistically significant.

Results

Of 98 pregnant women, 73 (74%) gave birth by cesarean section. Mean ± SD age of the pregnant women was 28.55 ± 5.47 (18–49) years. Forty-seven (48%) babies were female. Parities of women were 0–4. Forty-two percent of newborns with normal cord blood vit-B12 levels were the first live birth of family. There was no significant difference in the anthropometric measurements of newborns between the vit-B12 level groups, whereas the vit-B12 deficient group had larger heads than the marginal group too (Table 1).

Vit-B12 level was lower than 300 pg/mL in 93% of women and 61% of cord blood samples. There was a statistically significant relationship between serum vit-B12 levels of pregnant women and cord blood samples although the babies' vit-B12 levels were around 1.8 times higher than the respective maternal samples. No folate deficiency was found in either the pregnant women or cord blood. Data regarding hemograms, serum vit-B12, folate, and Hcy levels for women and cord blood are given in Table 2. No pathological Hcy level occurred in cord blood and only one pregnant woman had an Hcy concentration of 17.9 μmol/L (corresponding cord blood Hcy concentration 11.4 μmol/L). Further investigation of this mother revealed a heterozygous *MTHFR* gene mutation.

C3 carnitine concentrations slightly increased with decreasing vit-B12 concentration but this was not significant. Urine samples of 91 newborns those mothers' vit-B12 concentration was < 300 pg/mL were analyzed for MMA. In total 43 babies were excreting MMA. The highest urine MMA concentration was 53 mmol/mol-

Table 2. Comparison of hemogram parameters, serum B12, folate, and homocysteine levels between maternal and cord blood samples.

	Maternal blood mean ± SD	Cord blood mean ± SD	p
Hb (g/dL)	11.7 ± 1.3	14.6 ± 1.6	.176
Hct (%)	35.5 ± 5.0	44.5 ± 4.7	.954
MCV (fL)	88.0 ± 7.1	105.4 ± 4.7	.194
MCH (pg)	28.7 ± 2.5	34.6 ± 1.8	.510
B12 (pg/mL)	170.2 ± 87.7	313.3 ± 203.3	**<.001**
Folate (ng/mL)	15.5 ± 4.7	19.6 ± 1.4	**<.001**
Hcy (μmol/L)	5.6 ± 1.9	5.1 ± 1.6	**<.001**

SD: standard deviation; Hb: hemoglobin; Hct: hematocrit; MCV: mid corpuscular volume; MCH: mid corpuscular hemoglobin; Hcy: homocysteine. Bold represents significant $p < .05$.

crea. There was no MMA excretion in the urine samples of 27 babies with serum vit-B12 concentration < 300 pg/mL in cord blood and their mothers. The relationships between pregnant women and cord blood serum vit-B12 concentrations (divided into < 200, 200–300, and >300-pg/mL groups) and hemogram, serum vit-B12, folate, Hcy, baby C3 carnitine, and baby urine MMA values are summarized in Table 3.

Consumption of multivitamin supplements containing around 4 μg (range 2.5 mcg–1 mg) vit-B12 was reported by 76 (77.5%) pregnant women. Folic acid supplement (0.5–5 mg) intake was reported by 67 (68%) pregnant women. Mothers' folic acid consumption was significantly related to mother's serum folate level. Consumption of multivitamin preparations containing vit-B12 was also significantly related to newborn's serum vit-B12 level, pregnant women's folate and hemogram parameters (Table 4). None of the pregnant women were vegetarian.

Correlation analyses

There were statistically significant correlations between the maternal serum folate-B12 and cord blood folate-B12. The correlation analyses between pregnant women and cord blood folate-B12 levels and other parameters are given in Table 5.

Regression analysis

Using backward stepwise linear regression model, maternal serum vit-B12 concentration was found as the factor with the greatest effect on cord blood vit-B12 concentration (adjusted $R^2 = 0.453$) (Table 6).

ROC curve analysis

A cutoff value for cord blood vit-B12 concentration according to C3 carnitine (cutoff value = 3.16 μmol/L) was 232.5 pg/mL ($p = .014$) (Figure 1). ROC analysis for cord blood serum vit-B12 concentration according to newborn urine MMA was insignificant.

ROC analysis was performed separately for C3 carnitine, serum Hcy and urine MMA using a cord blood vit-B12 cutoff value of 200 pg/mL. This was not significant for C3 carnitine due to low sensitivity rate. However, Hcy cutoff value was found to be 4.77 μmol/L (68% sensitivity, 58% specificity, $p = .012$) (Figure 2). ROC analysis for urine MMA by newborn serum vit-B12 was again not significant.

Discussion

In the present study, we have shown that, even though serum folate levels were sufficient, vit-B12 deficiency was very common among pregnant women (93%) and fairly common in newborns (61%) despite easy access to health institutions in Istanbul. Although there are articles reporting low serum vit-B12 and folate levels in pregnant women causing low birth weight [2], there are also studies stating no correlation between vit-B12 level and anthropometric measurements [10]. In our study, we did not find lower birth weight in babies with low vit-B12 and neither was birth length affected. However, subjects in the marginal zone had lower values for head circumference than the deficient group. This difference is probably due to our relatively small sample size and may be a coincidental finding. Marginal zone should be checked for head circumference with a larger sample size.

In agreement with the existing evidence, our study showed that the most important factor determining vit-B12 concentration in cord blood was maternal vit-B12 concentration [11]. There was also a significant correlation between maternal and neonatal serum vit-B12 concentrations and they were corelevant. The cord blood vit-B12 concentration was 1.8 times greater than maternal concentration. Adaikalakoteswari et al. [12] reported cord blood vit-B12 concentrations 1.4 times higher than maternal values. A study conducted by Jacquemyn et al. [13] showed the same correlation between maternal and cord blood vit-B12 concentrations in which cord blood values were higher again. Another point of interest in the same study was the detection of deficiency, even in the 75.6% of women who reported taking vitamin supplements. Similarly, we also determined deficiency in a substantial proportion of our maternal subjects, despite taking vitamin supplements. However, there was a notable positive impact of maternal use of vit-B12 on cord blood vit-B12 concentrations. We suggest that a reevaluation of the amount of vit-B12 given to pregnant women is warranted.

We found a significant correlation between maternal folate-B12 and newborn folate-B12 serum concentrations. It is known that high levels of folate can mask vit-B12 deficiency [14]. Therefore, it should be kept in mind that the high levels of folate found in our study might also mask higher rates of vit-B12 deficiency, leaving neonates at a higher risk for neurologic development.

Hematologic manifestations might be absent in vit-B12 deficiency [15]. Our study specifically included iron-replete pregnant women and there was still no

Table 3. Comparison of maternal and cord blood hemogram, B12, folate, Hcy levels with normal, marginal, and low vitamin B12 levels in maternal and cord blood.

	Maternal serum B12 level (pg/mL)				Cord blood serum B12 level (pg/mL)			
	< 200 ($n = 72$)	200–300 ($n = 19$)	> 300 ($n = 7$)	p	< 200 ($n = 25$)	200–300 ($n = 35$)	> 300 ($n = 38$)	p
Maternal blood								
Hb (g/dL)	11.6 ± 1.3	12.0 ± 1.1	12.0 ± 1.2	.426	11.3 ± 1.3	11.6 ± 1.3	12.1 ± 1.2	**.046**
Hcy (%)	35.6 ± 3.9	34.8 ± 8.4	36.8 ± 3.9	.628	34.5 ± 3.8	35.6 ± 3.6	36.1 ± 6.6	.472
MCV (fL)	87.4 ± 7.2	89.1 ± 6.5	90.6 ± 6.9	.411	86.3 ± 5.5	87.1 ± 7.8	89.9 ± 6.9	.091
MCH (pg)	28.5 ± 2.6	29.2 ± 2.3	29.5 ± 2.7	.391	28.1 ± 2.1	28.5 ± 2.8	29.3 ± 2.4	.135
B12 (pg/mL)	136.8 (50.2–199.4)	232.9 (202.9–292.7)	384.9 (324.3–540.0)	**<.001***	111.8 (60.8–540.0)	138.4 (50.2–337.9)	198.0 (54.0–232.9)	**<.001**
Folate (ng/mL)	15.5 ± 4.6	15.1 ± 5.2	17.2 ± 4.6	.598	14.4 ± 4.8	15.7 ± 4.5	16.2 ± 4.8	.342
Hcy (µmol/L)	5.7 ± 2.1	4.9 ± 1.2	6.2 ± 1.4	.185	5.8 ± 1.5	5.8 ± 2.7	5.3 ± 1.8	.536
Cord blood								
Hb (g/dL)	14.50 ± 1.5	14.6 ± 1.9	15.6 ± 0.9	.229	13.7 ± 1.1	14.8 ± 1.7	15.0 ± 1.6	**.003**
Hcy (%)	44.14 ± 4.5	44.5 ± 5.6	47.7 ± 3.2	.157	41.9 ± 3.4	45.1 ± 4.7	45.6 ± 4.8	**.004**
MCV (fL)	105.01 ± 5.1	106.1 ± 3.3	107.3 ± 3.3	.360	105.1 ± 6.7	105.3 ± 3.4	105.7 ± 4.2	**.032**
MCH (pg)	34.50 ± 1.9	35.0 ± 1.4	35.0 ± 1.5	.528	34.5 ± 2.5	34.6 ± 1.5	34.7 ± 1.6	.057
B12 (pg/mL)	229.3 (98.8–800.4)	364.3 (141.2–698.2)	510.7 (253.8–885.9)	**<.001**	141.2 (98.8–198.5)	234.4 (200.5–294.3)	377.8 (301.2–885.9)	**<.001**
Folate (ng/mL)	20 (12.5–20.0)	20 (13.8–20.0)	20 (20.0–20.0)	.690	20 (13.4–20.0)	20 (17.8–20.0)	20 (12.5–20.0)	.213
Hcy (µmol/L)	5.4 ± 1.6	4.5 ± 1.3	4.0 ± 1.0	**.009**	5.6 ± 1.6	5.3 ± 1.8	4.7 ± 1.1	**.044**
Baby heel blood								
C3 carnitine (µmol/L)	2.8 ± 1.3	2.6 ± 1.02	2.5 ± 0.7	.687	3.3 ± 1.5	2.6 ± 1.1	2.7 ± 0.9	.148*
Baby's urine								
MMA n (%) > 0 mmol/mol-crea	36	6	0	.189	14	18	10	.121

Hb: hemoglobin; Hcy: hematocrit; MCV: mid corpuscular volume; MCH: mid corpuscular hemoglobin; Hcy: homocysteine; C3 carnitine: propionyl carnitine; MMA: methymalonic acid.
*p values were found by the Kruskal–Wallis analysis.
Bold represents significant $p < .05$.

Table 4. Comparison of hemogram parameters, B12, folate, Hcy, and baby C3 carnitine levels in pregnant women and cord blood according to the consumption of B12 and folate supplements.

	Consumption of B12 containing supplements			*Consumption of folate containing supplements*		
	Yes ($n = 76$)	No ($n = 22$)	*p*	Yes ($n = 67$)	No ($n = 31$)	*p*
Mother Hb	12.0 ± 1.2	11.0 ± 1.1	**.001**	11.9 ± 1.3	11.5 ± 1.3	.141
Cord Hb	14.6 ± 1.6	14.6 ± 1.6	.990	14.6 ± 1.6	14.5 ± 1.5	.704
Mother HCT	36.2 ± 5.3	33.4 ± 2.9	**<.001**	35.8 ± 5.5	35.0 ± 3.6	.472
Cord HCT	44.5 ± 4.7	44.3 ± 4.7	.931	44.7 ± 4.6	44.0 ± 4.8	.480
Mother MCV	89.3 ± 6.7	83.7 ± 6.8	**.001**	88.8 ± 7.0	86.3 ± 7.0	.114
Cord MCV	105.1 ± 4.9	106.0 ± 3.6	.397	104.9 ± 5.1	106.5 ± 3.6	.104
Mother MCH	29.2 ± 2.4	27.2 ± 2.5	**.001**	29.0 ± 2.5	28.1 ± 2.5	.100
Cord MCH	34.5 ± 1.9	34.9 ± 1.4	.394	34.4 ± 1.9	35.1 ± 1.4	.049
Mother B12	174.7 ± 91.2	157.8 ± 76.2	.587	173.3 ± 95.6	163.6 ± 68.5	.614
Cord B12	338.0 ± 216.4	238.3 ± 125.5	**.019**	319.9 ± 215.8	299.0 ± 175.6	.637
Mother folate	16.4 ± 4.3	12.5 ± 5.0	**.001**	16.8 ± 3.9	12.7 ± 5.1	**<.001**
Cord folate	19.7 ± 1.2	19.2 ± 1.9	.239	19.7 ± 1.1	19.3 ± 1.8	.261
Mother Hcy	5.4 ± 1.4	6.2 ± 3.0	.389	5.5 ± 1.5	5.8 ± 2.6	.413
Cord Hcy	4.9 ± 1.4	5.8 ± 2.0	.053	5.1 ± 1.5	5.2 ± 1.9	.696
Baby C3 carnitine	2.7 ± 1.1	3.1 ± 1.4	.097	2.7 ± 1.2	2.8 ± 1.2	.808

Hb: hemoglobin; Hcy: hematocrit; MCV: mid corpuscular volume; MCH: mid corpuscular hemoglobin; Hcy: homocysteine.
Bold represents significant $p < .05$.

Table 5. Correlation analyses of maternal and cord blood serum B12 and folate levels with other parameters.

	Maternal blood				Cord blood			
	B12[a]		Folate		B12[a]		Folate[a]	
	r	*P*	*r*	*p*	*r*	*p*	*r*	*p*
Mother Hb	0.266	**.008**	0.300	**.003**	0.300	**.003**	−0.013	.898
Cord Hb	0.185	**.068**	0.037	.716	0.279	**.005**	0.240	**.018**
Mother Hct	0.180	.076	0.133	.193	0.253	**.012**	−0.055	.589
Cord Hct	0.197	.052	0.030	.766	0.277	**.006**	0.197	.052
Mother MCV	0.181	.075	0.309	**.002**	0.319	**.001**	0.015	.880
Cord MCV	0.057	.575	0.052	.614	0.001	.990	0.090	.377
Mother MCH	0.212	**.037**	0.337	**.001**	0.332	**.001**	0.045	.662
Cord MCH	0.061	.553	0.040	.693	0.005	.964	0.146	.150
Mother B12	–	–	0.177	.081	0.562	**<.001**	0.206	**.041**
Cord B12	0.562	**<.001**	0.230	**.023**	–	–	0.200	**.049**
Mother folate	0.209	**.039**	–	–	0.209	**.039**	0.402	**<.001**
Cord folate	0.206	**.041**	0.402	**<.001**	0.200	**.049**	–	–
Mother Hcy	−0.066	.517	−0.076	.456	−0.113	.269	−0.120	.237
Cord Hcy	−0.258	**.010**	−0.201	**.047**	−0.308	**.002**	−0.223	**.027**
Baby C3 carnitine	−0.118	.247	−0.126	.216	−0.220	**.029**	−0.265	**.008**
Baby urine MMA	−0.126	.232	0.038	.721	−0.182	.084	0.099	.353

[a]Spearman correlation analysis was used for these nonparametric parameters. Hb: hemoglobin; Hcy: hematocrit; MCV: mid corpuscular volume; MCH: mid corpuscular hemoglobin; Hcy: homocysteine.
Bold represents significant $p < .05$.

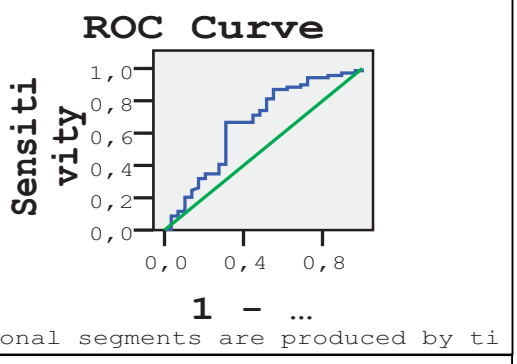
Table 6. Backward stepwise linear regression analyses: factors independently associated with cord blood vitamin B12 level (adjusted $R^2 = 0.453$).

	B	SE	*t*	*p*	OR (95% CI)
Maternal vitamin B12 level	1.569	0.174	9.009	**<.001**	1.223–1.915

Dependent variable cord serum B12 level; independent variables mother's age, B12 and folate level, baby's weight, Hcy and C3 carnitine levels. Significance $p < .05$.
Bold represents significant $p < .05$.

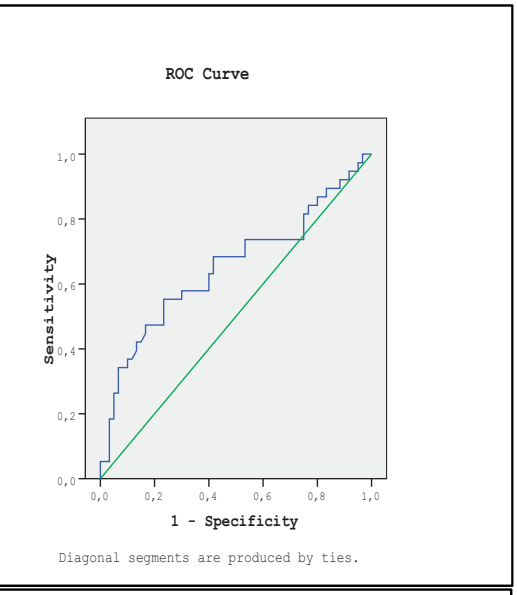
significant relationship between vit-B12 and MCV/MCH, despite the fact that some were severely vit-B12 deficient. So, megaloblastic changes may not be observed in vit-B12 deficient mothers and newborns. However, many studies have shown that neurologic deficits may appear before or without the development of megaloblastic changes. For example, Lindenbaum et al. [15] investigated 141 neurologically problematic patients due to vit-B12 deficiency and 28% had findings of neurological impairment in the absence of macrocytosis or anemia.

Clinical symptoms related to vit-B12 deficiency are usually nonspecific and difficult to detect, especially in newborns and infants. Vit-B12 deficient children may present with severe neurologic sequelae [16–18]. Neurological symptoms may occur even at normal levels of serum vit-B12. For this reason, diagnosis of vit-B12 deficiency should be made robustly. In our



AUC: 65.8%, SE: 0.065, p=0.014, 95% CI: 0.531-0.784, serum B12 cut-off value: 232.5 pg/mL, 66.7% sensitivity, 69% specificity

Figure 1. ROC curve of serum vit-B12 level according to baby C3 carnitine level of 3.16 μmol/L. Sensitivity (*Y* axis) is plotted against the specificity (*X* axis).



AUC: 65.1%, SE: 0.060, p=0.012, 95% CI: 0.533-0.769, serum Hcy cut-off value: 4.77 μmol/L, 68% sensitivity, 58% specificity

Figure 2. ROC curve of serum Hcy according to serum vit-B12 cut-off level recommended by WHO (221 pmol/L). Sensitivity (*Y* axis) is plotted against the specificity (*X* axis).

opinion, it is not sufficient to simply measure serum vit-B12 concentration, but vit-B12 related metabolites should also be measured which better reflect tissue vit-B12. Evidence shows that vit-B12 deficiency causes elevated levels of serum Hcy, C3 carnitine, and urine MMA [5,14,19–22]. Solomon et al. [23] detected concentrations of MMA and/or Hcy of >3SDs in almost all patients who were clinically and laboratory vit-B12 deficient. They suggested measuring serum vit-B12 concentration as a first step. Then, if serum vit-B12 was low, near the limit or normal but with clinical suspicion of vit-B12 deficiency, serum Hcy, and serum/urine MMA should be evaluated as a secondary examination. In contrast, Herrman et al. [21] proposed measurement of holo-transcobalamin and MMA. In some studies, C3 carnitine levels were obtained from extended newborn screening tests using blood spot samples [5,24], however abnormal results on screening must be confirmed by more accurate diagnosis. In our study, we evaluated vit-B12 deficiency with folate, Hcy, C3 carnitine, and urine MMA levels together and this highlights our work.

Some studies in the literature have accepted values below 200–300 pg/mL (marginal zone) as vit-B12 deficiency, whereas others did not specify whether they took undetermined intervals or reference values lower limit as a cutoff value for deficiency [9]. Rosenblatt et al. [25] reported serum vit-B12 level as < 200 ng/L in patients with clinical findings and 250–350 ng/L in patients with elevated Hcy and MMA levels without clinical findings. We aimed to designate a new cutoff value for vit-B12, specific to newborns, by measuring vit-B12-related metabolites. Our analysis showed a reliable value of 232.5 pg/mL with approximately 70% sensitivity and specificity rates, according to newborn C3 carnitine levels. We could not analyze it further in relation to Hcy because of the absence of pathological cord blood Hcy concentrations in our subjects. When we investigated the same relationship with urine MMA this resulted in a nonsignificant cutoff value. We believe that the serum vit-B12 cutoff value specific to newborns using vit-B12-related metabolites should be investigated with larger patient populations.

The Hcy cutoff value used in vit-B12 deficiencies is usually 15 μmol/L [9]. Bailey et al. [26] found upper limit of Hcy to be 10 μmol/L (1.35 μg/mL) in children younger than 1-year-old. Altuntaş et al. [27] designated normal serum Hcy level as 3.87 ± 1.44 μmol/L in 0–6-year-old children. In our study, we found 4.77 μmol/L of Hcy as a cutoff value with 68.4% sensitivity and 58.3% specificity in serum vit-B12 deficient (<300 pg/mL) newborns. We believe more research should be performed in this regard because elevated serum Hcy due to vit-B12 deficiency has different cutoff values in newborns.

Fletcher [24] reported frequency of C3 carnitine elevation to be 0.15% in newborn screening of a South Australian population and the most common cause was vit-B12 deficiency. Sarafoglou et al. [28] successfully detected vit-B12 deficiency in 11 patients using

lower cutoff values for C3 carnitine, urine MMA, and serum Hcy levels. According to our results, C3 carnitine had negative correlations with cord blood folate and vit-B12 levels. However, we could not find a significant difference between groups or a significant cutoff value by ROC analysis. More research with larger sample sizes is again needed to determine a reliable value for C3 carnitine in vit-B12 deficient newborns.

Kalay et al. [29] proposed the assessment of MMA excretion in urine as the most sensitive test for the detection of vit-B12 deficiency. Similarly, Scolamiero et al. [5] claimed MMA as a more specific marker, because the authors found excretion of MMA in urine without elevation of C3 carnitine in newborns with vit-B12-deficient mothers. In our study, newborns with low serum vit-B12 levels without MMA excretion in urine demonstrated that MMA urine testing alone was not sufficient for diagnosis. However, our urine MMA results may not be reliable because the sensitivity of the test used in our study was lower than some other current methods. Also, it should not be forgotten that serum MMA testing is more accurate than urine [30].

Conclusion

Vitamin B12 deficiency in pregnant women and newborns is prevalent and, therefore, still an important health issue. Our study revealed a lower and reliable cutoff value for serum Hcy in newborns for detection of nutritional vit-B12 deficiency. Also, it suggested a new cutoff value of 232.5 pg/mL for serum vit-B12 level in this specific population. Another point of interest is the high frequency of vit-B12 deficiency in mothers who reported using B12 containing multivitamin supplements. It is necessary to reevaluate the amount of vit-B12 used during pregnancy by studies conducted with larger sample size.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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